



#5

<110> Maxygen ApS

Maxygen Holdings Ltd.

<120> Single-Chain Polypeptides

<130> 0218us210

<150> US 60/245,727

<151> 2000-11-02

<160> 16

<170> PatentIn version 3.1

<210> 1

<211> 174

<212> PRT

<213> Homo sapiens

<400> 1

Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys
1 5 10 15

Cys Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln
20 25 30

Glu Lys Leu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu Glu Leu Val
35 40 45

Leu Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser Cys
50 55 60

Pro Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His Ser
1

65		70		75		80									
Gly	Leu	Phe	Leu	Tyr	Gln	Gly	Leu	Leu	Gln	Ala	Leu	Glu	Gly	Ile	Ser
				85					90					95	
Pro	Glu	Leu	Gly	Pro	Thr	Leu	Asp	Thr	Leu	Gln	Leu	Asp	Val	Ala	Asp
			100					105					110		
Phe	Ala	Thr	Thr	Ile	Trp	Gln	Gln	Met	Glu	Glu	Leu	Gly	Met	Ala	Pro
		115					120					125			
Ala	Leu	Gln	Pro	Thr	Gln	Gly	Ala	Met	Pro	Ala	Phe	Ala	Ser	Ala	Phe
	130					135					140				
Gln	Arg	Arg	Ala	Gly	Gly	Val	Leu	Val	Ala	Ser	His	Leu	Gln	Ser	Phe
145					150					155					160
Leu	Glu	Val	Ser	Tyr	Arg	Val	Leu	Arg	His	Leu	Ala	Gln	Pro		
				165					170						

<210> 2
 <211> 63
 <212> DNA
 <213> *Saccharomyces cerevisiae*

<400> 2	
atgaaattga aaactgtag atctgctggt ttgtcttctt tgtttgcttc tcaagttttg	60
ggt	63

<210> 3
 <211> 126
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> leader sequence

<400> 3	
caaccaattg atgatactga atctcaaact acttctgtta atttgatggc tgatgatact	60
gaatctgctt ttgctactca aactaattct ggtgggtttgg atgttggttg tttgatatcg	120

atggcc

126

<210> 4

<211> 522

<212> DNA

<213> Artificial Sequence

<220>

<223> DNA encoding G-CSF copy 1 in the single chain G-CSF dimer

<400> 4

actccattgg gtccagcttc ttctttgcc caatcttttt tgttgaaatg tttggaacaa	60
gttagaaaaa ttcaaggtga tggctgctgt ttgcaagaaa aattgtgtgc tacttataaa	120
ttgtgtcatc cagaagaatt ggttttggtt ggtcattctt tgggtattcc atgggctcca	180
ttgtcttctt gtccatctca agctttgcaa ttggctgggt gtttgtctca attgcattct	240
ggtttggttt tgtatcaagg tttgttgcaa gctttggaag gtatttctcc agaattgggt	300
ccaactttgg atactttgca attggatggt gctgattttg ctactactat ttggcaacaa	360
atggaagaat tgggtatggc tccagctttg caaccaactc aagggtgctat gccagctttt	420
gcttctgctt ttcaaagaag agctgggtgt gttttggttg cttctcattt gcaatctttt	480
ttggaagttt cttatagagt ttgagacat ttggctcaac ca	522

<210> 5

<211> 531

<212> DNA

<213> Artificial Sequence

<220>

<223> DNA encoding G-CSF copy 2 in the single chain G-CSF dimer

<400> 5

accctctgg gcccgccag cagtctgcct cagagttttt tactgaaatg cttagaacag	60
gtgcgtaaaa tccagggcga tggcgggcc ctgcaggaaa aactgtgcgc gacctataaa	120
ctgtgccatc ctgaagaact ggtcctgtta ggccatagct taggcatccc gtgggcgcct	180
ctgagtagct gcccgagtca ggccctgcag ctggccggct gcctgagtca gttacatagt	240

ggcttatttt tatatcaggg cttactgcag gcgttagaag gcattagtcc ggaactgggc 300
 ccgaccctgg atacccttaca gttagatgtc gcggattttg ccaccaccat ttggcagcag 360
 atggaagaat taggcatggc gcctgcgtta cagcctaccc agggcgccat gcctgcgttt 420
 gcgagtgcgt ttcagcgtcg cgccggcggc gtgttagtgg ccagccatct gcagagcttt 480
 ctggaagtga gttatcgtgt gttacgccat ctggcccagc cttaatctag a 531

<210> 6

<211> 348

<212> PRT

<213> Artificial Sequence

<220>

<223> Single chain G-CSF dimer polypeptide

<400> 6

Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys
 1 5 10 15

Cys Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln
 20 25 30

Glu Lys Leu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu Glu Leu Val
 35 40 45

Leu Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser Cys
 50 55 60

Pro Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His Ser
 65 70 75 80

Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile Ser
 85 90 95

Pro Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala Asp
 100 105 110

Phe Ala Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Met Ala Pro
 115 120 125

Ala Leu Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala Phe
 130 135 140

Gln Arg Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser Phe
145 150 155 160

Leu Glu Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro Thr Pro
165 170 175

Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys Cys Leu
180 185 190

Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln Glu Lys
195 200 205

Leu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu Glu Leu Val Leu Leu
210 215 220

Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser Cys Pro Ser
225 230 235 240

Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His Ser Gly Leu
245 250 255

Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile Ser Pro Glu
260 265 270

Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala Asp Phe Ala
275 280 285

Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Met Ala Pro Ala Leu
290 295 300

Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala Phe Gln Arg
305 310 315 320

Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser Phe Leu Glu
325 330 335

Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro
340 345

<210> 7

<211> 90

<212> DNA

<213> Homo sapiens

<400> 7
atggctggac ctgccacca gagcccatg aagctgatgg ccctgcagct gctgctgtgg 60
cacagtgcac tctggacagt gcaggaagcc 90

<210> 8

<211> 522

<212> DNA

<213> Artificial Sequence

<220>

<223> DNA encoding single-chain G-CSF copy 1 (codon usage optimized for expression in CHO cells)

<400> 8
actccattgg gtccagcttc ttctttgcca caatcttttt tgttgaaatg tttggaacaa 60
gttagaaaaa ttcaaggtga tgggtgctgct ttgcaagaaa aattgtgtgc tacttataaa 120
ttgtgtcatc cagaagaatt ggttttggtg ggtcattctt tgggtattcc atgggctcca 180
ttgtcttctt gtccatctca agctttgcaa ttggctgggt gtttgtctca attgcattct 240
ggtttgtttt tgtatcaagg tttgttgcaa gctttggaag gtatttctcc agaattgggt 300
ccaactttgg atactttgca attggatggt gctgattttg ctactactat ttggcaacaa 360
atggaagaat tgggtatggc tccagctttg caaccaactc aagggtgctat gccagctttt 420
gcttctgctt ttcaaagaag agctgggtgt gttttgggtg cttctcattt gcaatctttt 480
ttggaagttt cttatagagt tttgagacat ttggctcaac ca 522

<210> 9

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<223> tag

<400> 9

His His His His His His

1 5

<210> 10

<211> 8

<212> PRT

<213> Artificial Sequence

<220>

<223> tag

<400> 10

Met Lys His His His His His His
1 5

<210> 11

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> tag

<400> 11

Met Lys His His Ala His His Gln His His
1 5 10

<210> 12

<211> 14

<212> PRT

<213> Artificial Sequence

<220>

<223> tag

<400> 12

Met Lys His Gln His Gln His Gln His Gln His Gln His Gln
1 5 10

<210> 13

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> tag

<400> 13

Met Lys His Gln His Gln His Gln His Gln His Gln His Gln Gln
1 5 10 15

<210> 14

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> tag

<400> 14

Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu
1 5 10

<210> 15

<211> 8

<212> PRT

<213> Artificial Sequence

<220>

<223> tag

<400> 15

Asp Tyr Lys Asp Asp Asp Asp Lys
1 5

<210> 16

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> tag

<400> 16

Tyr Pro Tyr Asp Val Pro Asp Tyr Ala
1 5

1